Brief Agency summaries of MRID numbers 00087417, 00087428, 00151228, 00144940, 00144098, 00145267,00145869 and 40869201

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: July 17, 1979

074901

SUBJECT: Neurotoxic Review of DEF and Merphos

FROM: Pharmacologist, Toxicology Branch/HED (TS-769)

Bipin Ghandi

TO: PM, SPRD (TS-791)

I have reviewed the documents which you have given me on the neurotoxicity of DEF and Merphos and the reviews are attached. As a result of this review, I am convinced that there are significant differences in the toxicity of these two compounds. The differences are such that under no circumstances should information on one compound be used in a report on the other compound.

I have identified three items of concern with DEF, at least two and possibly three of these are also present in merphos. The items are 1) delayed neurotoxicity, 2) histopathological damage to the spinal cord at doses which do not produce clinical signs and 3) extreme veriability in the effectiveness of treatment of the acute toxic effects.

The delayed neurotoxicity, by several routes of administration, is clearly established with both compounds but there are differences in route-response relatinships with the two compounds. We have a clear-cut human case of Merphos produced delayed paralysis, which was fortunately reversible in time, that gives me some doubt that the toxic effect is the "classical" delayed neurotoxic syndrome.

The histopathological lesions reported by Baron and Johnson following oral administration are puzzling. They have all the signs of a real observation yet Baron has been unable to reproduce them and now believes they were an artifact. This may need some more looking at utilizing a variety of different straining methods.

The antidoting problem with DEF is a possible trigger that was not touched on previously and it certainly needs clearification. I have no information that this problem exists with Merphos but I am very suspicious. I suggest that you do a little digging in the records to see if any antidote to Merphos is mentioned and if there is any experimental basis for it.

I have reviewed the papers on possible carcinogenic, fetotoxic and teratogenic effects of DEF and Merphos. Since all of these researchers used DEF it is impossible to obtain any indication of the toxicity of Merphos and we would err seriously if we attempted to utilize them that way. The information is sufficient to allow us to ask for additional studies but I do not believe that it is sufficiently clear cut for RPAR purposes.

We will need carcinogeic, fetotoxic and teratogenic studies on both compounds by both oral and dermal route.

Robert Zendzian, Ph.D.

DEF

A review of the literature on neurological effects of DEF has identified three areas of toxicological concern. These are (1) an inexplicable variability in antidoting the acute toxic effects, (2) delayed neurotoxicity and (3) histopathological evidence of nerve damage without accompaning clinical signs.

Antidoting Acute Toxic Effects

DEF has been identified as a cholinesterase inhibiting agent and as such it should be possible to antidote its acute toxic effects by using an anticholinergic agent such as atropine. Addition of an enzyme reactavating agent such as PAM, can shorten the time until full recovery from the acute toxic effects of certain cholinesterase inhibiting agents. Thysson and Mohr (1976) reported that 25 of 30 hens pretreated with atropine 50 mg/kg and PAM 100 mg/kg died of the acute toxic effects of a single oral dose of 300 mg/kg DEF. The authors repeated this treatment on a fresh group of 10 hens and all survived. The dose of DEF was a calculated oral LD_{50} derived from a more than adequate oral toxicity study.

The authors were unable to explain their contradictory results. The DEF used was a technical grade sample and there is no indication that the same sample or batch was used for each study reported in the paper.

Murphy and DuBois (1959) reported on a series of studies on the toxicity of DEF following single or repeated doses to rats, mice and guinea pigs. Based on their observation of cholinesterase inhibition and signs of excessive cholinergic activation in the test animals the authors attempted to antidote the DEF toxicity with atropine.

"If the toxicity of DEF could be attributed solely to its ability to inhibit cholinesterase, it might be expected that atropine would be useful as an antidote. Experiments were, therefore, undertaken to test this possibility. The intraperitoneal administration of 100 mg per kilogram of atropine sulfate to female rats 15 minutes before the injection of DEF by the same route failed to protect against one LD_{50} (210 mg per kilogram) of the defoliant. When the same dose of atropine was given 15 minutes before 210 mg per kilogram of DEF and repeated at 4, 12, 24, and 36 hours after administration of the defoliant, no protection was observed. These experiments demonstrated the ineffectiveness of atropine as an antidote for DEF and provided further evidence that actions other than cholinesterase inhibition are important in poisoning by DEF."

Abou-Donia (1978) reported extremely strange results following atropine treatment of hens given 100 to 1000 mg/kg of DEF in a single oral dose.

"All hens treated with 100 mg/kg or more of DEF, were given 30 mg/kg of atropine sulfate for 4 days. All treated hens were active and appeared normal during the 24 hr. following the oral administration of DEF. By the beginning of the second day, however, most of the treated hens showed leg weakness, unsteadyness and secreted a yellowish watery fluid from the mouth. The severity of a toxic signs was dose dependent, and was more severe in those hens that were given the highest doses. As time passed, the bird's conditions deteriotated despite continuing treatment with 30 mg/kg atropin sulfate. The atropine sulfate dose was decreased to 20 and 10 mg/kg on the fifth and sixth day respectively, since it was noted that many birds died shortly after the administration of 30 mg/kg of atropine sulfate." Atropine at a dose of 300 mg/kg had no toxic effect on a group of hens acting as atropine controls. Abou-Donia attribution the acute oral toxicity of DEF to n-buthyl mercaptan which cannot be antidoted with atropine.

These studies indicate the possibility of variation in composition of the technical grade of DEF with a resultant veriation in mechanism of acute toxicity including a type of acute toxicity for which no antidote is known.

Delayed Neurotoxicity

The first indication that DEF could produce delayed neurotoxicity was reported by Casada et. al. in 1963. DEF was administered intraperitoneally in corn oil to White Leghorn Hens. "With the phosphate trithioate (Campt. 95 or DEF) at 100 mg/kg daily for 10 days ataxia appeared in 10 and 18 days after the treatment was started, while with 7 days of treatment at this dose, ataxia appeared on day 14."

In 1964 Baron & Johnson reported that multiple intraperitoneal injection of DEF in hens produced the pathological lesions and clinical signs of delayed neurotoxicity. Multipal oral administration produced pathological lesions but no clinical signs in the hens. By the peritoneal route doses of 50 mg/kg/day for 5, 10 or 15 days and of 100 mg/kg/day for 5, 10 or 15 days produced clinical signs and pathological lesions. By the oral route doses of 50 mg/kg/day for 5, 100 or 15 days and 100 mg/kg/day for 4 or 5 days produced pathological lesions but no clinical signs. One hen treated orally at 150 mg/kg for 5 days showed both clinical and patholgical signs of neurotoxicity.

In 1969 Gaines reported delayed neurotoxic effects induced by DEF in hens. The hens received a single oral dose of atropine 15mg/kg followed by a single subcutaneous dose of DEF in peanut oil at 200 mg/kg. Clinical signs of a neurological effect appeared 14 days after dosing.

Johnson reported in 1970 that a single subcutaneous dose of DEF produced neurotoxicity in hens. A specially purified preparation of DEF (greater than 99% pure) obtained from the Chemagro Crop was used in this study. A single dose of 220 mg/kg was inactive and a single dose of 1100 mg/kg was active.

Thysson and Mohr (1976) reported no delayed neurotoxicity following oral administration of DEF to hens. Technical grade DEF was emulsified in polyethylene glycol 400 and administered orally. Thirty hens was treated at the highest dose of 300 mg/kg, 25 died, and the five survivers did not show clinical or histopathological signs of delayed neurotoxicity.

Thysson and Schilde (1976) reported that a single dermal dose of technical grade DEF applied for 24 hours produced neurotoxic effects at doses of 1.0 and 2.0 ml/kg but not at 0.5 mg/kg. Since the specific gravity of DEF is 1.057 these three doses are approximately 1,004, 2,008 and 502 mg/kg.

Thysson and Schilde (1976b) reported the occurrence of delayed neurotoxicity in hens following exposure to DEF aerosols. A single four hour exposure of 1585 mg/m^3 DEF and five four hour exposures to 256 mg/m^3 DEF produced clinical and histological evidence of delayed neurotoxicity. Neurotoxicity was not observed after a single four hour exposure to 39 mg/m^3. The method of exposure was such that some DEF could have been absorbed through the skin or orally following preening.

In 1978 Abou-Donia reported the appearance of a delayed neurotoxic syndrome following acute and subchronic oral administration of DEF to hens. Technical grade DEF (91%) was administered orally in gelatin capsules. A single oral dose of 50 mg/kg had no toxic effect. Single oral dose of 100 through 1000 mg/kg produced clinical signs of delayed neurotoxicity. A subchronic oral dose of 0.1 mg/kg/day for 90 days produced no toxic effect while doses of 0.5 through 20 mg/kg/day for 90 days produced signs of delayed neurotoxicity. No histopathological changes indicative of DEF induced nerve damage was observed in this study.

In contrast to the above studies three feeding studies were uniformly negative.

Harris (1965a) reported that hens fed diets containing 0, 100, 250 and 500 ppm DEF for 60 days did not show clinical or histopathological signs of neurotoxicity.

Harris further reported (1965b) that no clinical or histopathological signs of delayed neurotoxicity occurred in hens diets containing 100, 200, 250 and 500 ppm for 30 days.

Thesson et. al. (1977) reported that hens feed diets containing DEF at doses of 0, 25, 50, 100, 200 and 400 ppm for 30 days followed by 30 days observation did not show clinical or histopathological signs of neurotoxicity.

The studies referenced above clearly demonstrate delayed neurotoxicity by oral, intraperitoneal, subcutaneous, dermal and inhalation routes. The negative oral study of Thysson and Mohr (1976) differed from the positive studies in that the DEF was emulsified in polyethylene glycol 400 as opposed to administration in corn oil or gelatin capsules in the positive studies. Differences between the results of feeding studies and oral administration are intrinsiclly harder to explain. The doses given were lower, spread out in time and given in a different vehicle (food).

Nerve Damage Without Clinical Signs

Baron and Johnson (1964) reported that oral administration of DEF to hens produced histopathological lesions in the spinal cord without producing clinical signs of damage. Intraperitoneal administration of the same material at the same dose regimen produced both clinical signs and histopathological damage.

Oral Doses of DEF, in corn oil of 50 mg/kg/day for 5, 10, or 15 days and 100 mg/kg/day for 4 or 5 days produced histopathological lesions in the spinal cord. "The pattern of disruption was not typical of that seen with other organophosphates. The damage was less localized and did not correspond to the specific tracts" observed in animals which received DEF intraperitoneally. The authors used the Swank-Davenport modification of the Marchi stain and were the only individuals studying DEF toxicity who reported using this stain.

In June 1977 Baron was contacted by telephone and asked if this particular observation had ever been followed-up. Baron stated that additional work has been done but he had never been able to duplicate the observation. He believes that the original stain observed was an artifact of the straining process. He is not aware of any other report of histopathological damage that was not accompanied by clinial signs and now believes that histopathological damage is always accompained by clinical signs.

A compound which produces histopathological damage to the nervous system without clinial signs in experimental animals represents a particularly dangerous type of toxicity. A "sick" chicken can be spotted by a trained observer while subclinical damage to the nervous system requires examination of the "right" tissue properly fixed, stained with the "right" stain and examined by a highly trained professional. Such damage is easy to miss by pure chance and it is too late when if occurs irreversibly in man.

We are left with the possibility, decidedly small, that Baron and Johnson's observation was not an artifact. The staining was bilateral, in an unexpected area of the spinal cord and not observed in the controls. The hens showing clinical signs were stained in the "right" or expected areas of the spinal cord.

Merphos

Two significant neurotoxic effects of merphos have been identified 1) delayed neurotoxicity and 2) histopathological evidence of damage to the spinal cord which is not accompained by clinical signs.

Delayed Neurotoxicity

In 1963 Casida et. al. reported that multiple daily intrapitoneal doses of Merphos produced a delayed neurotoxic neurotoxic syndrome in hens. "With the phosphorotrithioate analog (Compd 100 or Merphos) seven daily doses of 100 mg/kg gave ataxia on day 33 and ten daily doses of 100 mg/kg gave ataxia on day 25."

Baron and Johnson (1964) reported that, in hens, multipule intraperitoneal doses of Merphos in corn oil produced a delayed neurotoxic effect but oral doses did not. Intraperitoneal doses of 100 mg/kg/day for 5, 10 or 15 days produced a delayed neurotoxic effect at 10 and 15 days. Histopathological examination revealed distinct areas of damage in the spinal cord. Oral doses of 100, 150, 200 or 300 mg/kg/day for 10 days produced no signs of a delayed neurotoxic syndrome. However histopathological damage was observed in these hens. "An unusual result seen in the eight hens receiving Merphos administered orally for 10 days at dosages up to 300 mg/kg was the lack of muscular weakness coinciding with Marchi-positive strained sections of the spinal cord".

In 1969 Gaines reported that hens pretreated orally with 15 mg/kg of atropine showed delayed neurotoxic signs following a single subcutaneous dose of 600 mg/kg Merphos. "The time of onset of leg weakness in the treated chickens was delayed for at least 14 days following DEF and at least 3 days following Dursban and Merphos."

Babish (1977) reported that oral doses of Merphos up to 11 ml/kg (approximately 10,450 mg/kg) did not produce clinical signs of delayed neurotoxicity. Forty hens were treated orally and 15 died of the acute toxicity of Merphos. After 21 days the 25 survivors received a second dose and 11 died. Twenty-one days of observation failed to reveal signs of delayed neurotoxicity.

Cox and Babish reported in 1977 that 1 ml/kg of Merphos (approximately 950 mg/kg) applied dermally produced signs of delayed neurotoxicity. Forty hens were treated and two died. Signs of acute toxicity were observed during the first four days after dosing. Paralytic signs were observed approximately 14 days after dosing. Following histopathological examination of the spinal cord the authors reported that "6 animals occasionally demonstrated swelling of scattered fibers in the ventral furiculus of the spinal cord" which was similar in type to that seen in TOCP controls.

Fisher (1977) reported a delayed paralytic effect in a man who had spilled undiluted merphos while mixing the material prior to spraying. A healthy 28 year-old man accidently spilled a "moderate" amount of merphos on his bare upper arms and T-shirt, soaking the garment through to the skin. Six days after exposure he was hospitalized in a advanced stage of paralysis. Atropine and paralidoxime chloride were ineffective in reversing the paralysis. Six weeks after exposure he was released from the hospital but he required an additional 14 weeks for full recovery. Fisher was contracted by telephone and he stated that to the best of his knowlege the patient remains normal.

In 1977 Abou-Donia reported the appearance of a delayed neurotoxic syndrome following acute and subchronic oral administration of Merphos to hens. Technical grade Merphos (95%) was administered orally in gelatin capsules. A single dose at 100 mg/kg had no toxic effect. Single oral doses of 200 through 2000 mg/kg produced clinical signs of delayed neurotoxicity. A subchronic oral dose of 0.5 mg/kg/day for 90 days produced no toxic effects, while doses of 1.0 through 20 mg/kg/day for 90 days produced signs of delayed neurotoxicity. No histologic changes were seen in hens given single doses while hens given daily doses showed equivocal histopathological changes in the spinal cords of some hens.

The studies referenced above demonstrate delayed neurotoxicity by oral, intraperitoneal and dermal routes. The considerable diferences between oral route and the other routes may indicate a different pattern of drug metabolism in the production of an active cholinesterase inhibitor.

Nerve Damage Without Clinical Signs

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Chemical: ~~S,S,S-Tributyl phosphorotrithioate~~ Tribuphos; Phosphorotrithious Acid, S,S,S-tributyl ester

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